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(54) **PHARMACEUTICAL COMPOSITION FOR PREVENTING OR TREATING PRURITUS, CONTAINING PYRAZOLE DERIVATIVE AS ACTIVE INGREDIENT, AND SCREENING METHOD FOR DETECTING SAME**

(57) The present invention relates to a pharmaceutical composition for preventing or treating pruritus, containing a pyrazole derivative as an active ingredient, and a screening method for detecting the same. A pharmaceutical composition for preventing or treating pruritus, according to the present invention, can relieve the symptoms of pruritus by inhibiting the activity of intracellular Mrgpr X1, and can relieve the symptoms of pruritus by inhibiting the activity of intracellular hH1R, thereby being usable also as a medicine for preventing or treating histamine-mediated pruritus. In addition, it has been confirmed through dry skin mouse model experimentation that the composition also has significant alleviation effects on dry skin, thereby being usable as a medicine for treating dry skin. Furthermore, the pharmaceutical composition according to the present invention can relieve the symptoms of pruritus caused by psoriasis, thereby being usable also as a medicine for treating psoriasis. Moreover, the pharmaceutical composition according to the present invention can maintain stable activity, even during in vivo drug administration, without causing adverse reactions.

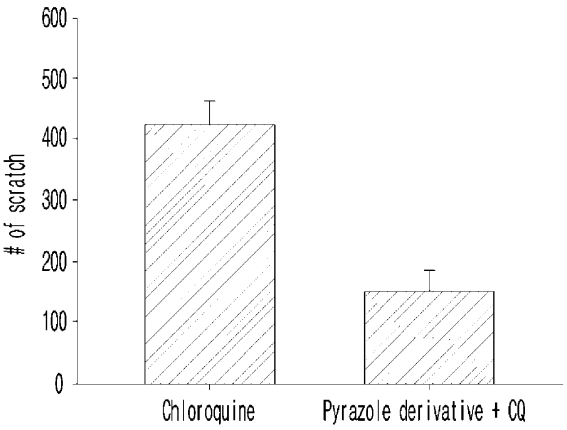

Fig.7

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Description

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0001] The present invention relates to a pharmaceutical composition for preventing or treating pruritus, containing a pyrazole derivative as an active ingredient, and a screening method for detecting the same.

2. Description of the Related Art

[0002] Pruritus (or itch) is a noticeable symptom appearing on whole body in various skin disease cases. Pruritus causes itching to protect the skin from bugs, toxic plants or other harmful stimuli. However, pruritus does not always play such a beneficiary role as the above. Chronic itch accompanies eczema, kidney disease, liver cirrhosis and skin diseases including some cancers. Many neurological diseases also cause severe itching, which are exemplified by including multiple sclerosis, diabetic neuropathy and postherpetic neuralgia (herpes zoster), etc. Pruritus is developed by sensory nerve cells and these cells are known to have a cell body in the dosal root ganglion.

[0003] It is known that various types of pruritus are mediated by histamine (histaminergic pruritus). Chloroquine, an antimalarial agent, is known to cause histamine-independent pruritus (non-histaminergic pruritus).

[0004] Mas-related G protein-coupled receptors (Mrgprs) are known as non-histaminergic pruritus receptors as GPCR (G protein-coupled receptors) expressed only in peripheral sensory neurons. These receptors are found in various tissues in adults, especially in neurons (Dong et al. 2001). Among them, Mrgpr X1 is distributed in the human dorsal root ganglion.

[0005] Histaminergic pruritus is known to be protected by human histamine receptor 1 antagonist (2009 Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. Cell 139, 1353-1365 (2009)). However, the antagonist has no effect on non-histaminergic pruritus. Allergic itch is mediated by histamine, which takes only one third of total pruritus. Thus, allergic itch can be treated with antihistamines, but most itches cannot be treated with antihistamines.

[0006] As a treatment agent for pruritus, preparations for cooling skin comprising calamine lotion or 1% menthol lotion, steroids, and antihistamines are currently used. However, those preparations for cooling skin have only a temporary effect. On the other hand, steroids demonstrate a strong antiinflammatory activity, so that they are excellent in alleviating the symptoms of various diseases such as joint disease, cerebrovascular disease, inflammatory disease and allergic disease, but are limited in use because of their side effects according to long term administration or overdose. Antihistamines are mainly formulated for oral administration. In general, drugs that inhibit only H1 receptor involved directly in pruritus have been developed. However, these drugs are also limited in use because they cause such side effects as overall decline of cognitive abilities and motor nerves. As explained above, there is no universal treatment method for pruritus, yet. Therefore, it is required to develop a safe and effective drug for pruritus.

[0007] Thus, the present inventors have studied to develop a safe and effective therapeutic agent which is not only effective in treating histaminergic pruritus but also effective in non-histaminergic pruritus. In the course of the study, the present inventors confirmed that a pyrazole derivative compound was effective in inhibiting the activities of Mrgpr X1 and hH1R (human histamine receptor subtype1), leading to the completion of the present invention.

SUMMARY OF THE INVENTION

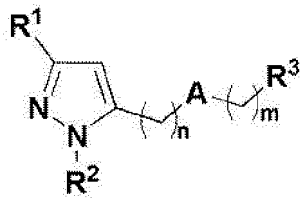
[0008] It is an object of the present invention to provide a pharmaceutical composition for preventing or treating pruritus.

[0009] It is another object of the present invention to provide a health functional food composition for preventing or treating pruritus.

[0010] It is also an object of the present invention to provide a screening method of a compound for preventing or treating pruritus.

[0011] To achieve the above objects, the present invention provides a pharmaceutical composition comprising a compound represented by formula 1 below or a pharmaceutically acceptable salt thereof as an active ingredient for preventing or treating pruritus.

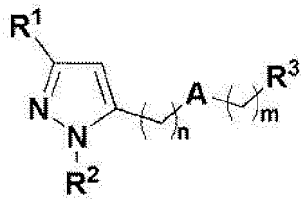
[Formula 1]



(In formula 1,
R^1, R^2, R^3, A, m and n are as defined in this specification.)

[0012] The present invention also provides a health functional food composition comprising a compound represented by formula 1 below or a pharmaceutically acceptable salt thereof as an active ingredient for preventing or alleviating pruritus.

[Formula 1]



(In formula 1,
R^1, R^2, R^3, A, m and n are as defined in this specification.)

[0013] Further, the present invention provides a screening method of a compound for preventing or treating pruritus, which comprises the following steps:

treating a pruritus-inducing substance to cells expressing MRGPR X1 (Mas-related G protein-coupled receptor), and culturing the cells (step 1); and
treating a candidate material to the pruritus-induced cells, and measuring the inhibition of MRGPR X1 activity (step 2).

[0014] In addition, the present invention provides a screening method of an active material for preventing or treating pruritus, which comprises the following steps:

treating a pruritus-inducing substance to cells expressing hH1R (human Histamine 1 Receptor), and culturing the cells (step 1); and
treating a candidate material to the pruritus-induced cells, and measuring the inhibition of hH1R activity (step 2).

ADVANTAGEOUS EFFECT

[0015] The pharmaceutical composition for preventing or treating pruritus according to the present invention can be effectively used as a preventive or therapeutic agent for non-histaminergic pruritus because it can relieve the symptoms of pruritus by inhibiting the activity of intracellular Mrgpr X1. The pharmaceutical composition for preventing or treating pruritus according to the present invention can be effectively used as a preventive or therapeutic agent for histaminergic pruritus because it can relieve the symptoms of pruritus by inhibiting the activity of intracellular hH1R. In addition, it has been confirmed through dry skin mouse model experimentation that the composition also has significant alleviation effects on dry skin, thereby being usable as a medicine for treating dry skin. Furthermore, the pharmaceutical composition according to the present invention can relieve the symptoms of pruritus caused by psoriasis, thereby being usable also as a medicine for treating psoriasis. Moreover, the pharmaceutical composition according to the present invention can maintain stable activity, even during in vivo drug administration, without causing adverse reactions.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016]

Figure 1 is a graph showing the results of fluorescence analysis performed after treating Mrgpr X1 cells with chloroquine alone.

Figure 2 is a graph showing the results of fluorescence analysis performed after treating Mrgpr X 1 cells with a pharmaceutical composition comprising the pyrazole derivative of Example 1 together with chloroquine.

Figure 3 is a graph showing the results of fluorescence analysis performed after treating hH1R cells with histamine alone.

Figure 4 is a graph showing the results of fluorescence analysis performed after treating hH1R cells with histamine and diphenhydramine.

Figure 5 is a graph showing the results of fluorescence analysis performed after treating hH1R cells with a pharmaceutical composition comprising the pyrazole derivative of Example 1 together with histamine.

Figure 6 is a graph illustrating the evaluation of pruritus treatment effect when the histamine mouse model is treated with a pharmaceutical composition comprising the pyrazole derivative of Example 1.

Figure 7 is a graph illustrating the evaluation of pruritus treatment effect when the chloroquine mouse model is treated with a pharmaceutical composition comprising the pyrazole derivative of Example 1.

Figure 8 is a graph illustrating the evaluation of pruritus treatment effect when the dry skin mouse model is treated with a pharmaceutical composition comprising the pyrazole derivative of Example 1.

Figure 9 is a graph showing the results of Rota rod motion condition test with the mouse treated with a pharmaceutical composition comprising the pyrazole derivative of Example 1.

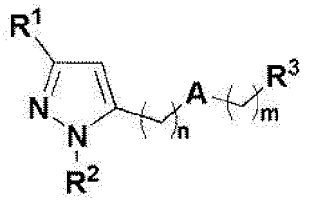
Figure 10 is a graph illustrating the evaluation of pruritus treatment effect when the psoriatic mouse model is treated with a pharmaceutical composition comprising the pyrazole derivative of Example 1.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0017] Hereinafter, the present invention is described in detail.

[0018] The present invention provides a pharmaceutical composition comprising a compound represented by formula 1 below or a pharmaceutically acceptable salt thereof as an active ingredient for preventing or treating pruritus.

[Formula 1]



[0019] In formula 1 above,

R^1 and R^2 are independently straight or branched C_1-C_6 alkyl, straight or branched C_1-C_6 alkoxy, -NO_2,-NR^4R^5 or nonsubstituted or substituted C_6-C_{12} aryl, wherein the substituted C_6-C_{12} aryl can be substituted with one or more substituents selected from the group consisting of halogen, straight or branched C_1-C_6 alkyl, straight or branched C_1-C_6 alkoxy, -NO_2 and-NR^4R^5, at this time, R^4 and R^5 are independently hydrogen or straight or branched C_1-C_6 alkyl;

R^3 is hydrogen, -(C=O)OR^6 or nonsubstituted or substituted 3-10 membered heterocycloalkyl or heterocycloalkenyl containing one or more heteroatoms selected from the group consisting of N, O and S, at this time, R^6 can be hydrogen or straight or branched C_1-C_6 alkyl, wherein the substituted heterocycloalkyl or heterocycloalkenyl can be substituted with one or more substituents selected from the group consisting of halogen, straight or branched C_1-C_6 alkyl and straight or branched C_1-C_6 alkoxy;

A is -NH-, -O-, -S-, -(C=O)NH-, -NH(C=O)-,-(C=O)O- or -O(C=O)-; and

m and n can independently be integers of 0-8.

[0020] In addition, in formula 1 above,

R^1 and R^2 are independently straight or branched C_1-C_3 alkyl, straight or branched C_1-C_3 alkoxy, -NO_2, - NR^4R^5 or nonsubstituted or substituted C_6-C_{10} aryl, wherein the substituted C_6-C_{10} aryl can be substituted with one or more substituents selected from the group consisting of halogen, straight or branched C_1-C_3 alkyl, straight or branched C_1-C_3 alkoxy, -NO_2 and-NR^4R^5, at this time, R^4 and R^5 are independently hydrogen or straight or branched C_1-C_3

alkyl;
R^3 is hydrogen, -(C=O)OR^6 or nonsubstituted or substituted 3-8 membered heterocycloalkyl or heterocycloalkenyl containing one or more heteroatoms selected from the group consisting of N, O and S, at this time, R^6 can be hydrogen or straight or branched C_1-C_3 alkyl, wherein the substituted heterocycloalkyl or heterocycloalkenyl can be substituted with one or more substituents selected from the group consisting of halogen, straight or branched C_1-C_3 alkyl and straight or branched C_1-C_3 alkoxy;
A is -NH-, -O-, -S-, -(C=O)NH-, -NH(C=O)-,-(C=0)0- or -O(C=O)-; and
m and n can independently be integers of 0-6.

[0021] Further, in formula 1 above,

R^1 and R^2 are independently straight or branched C_1-C_3 alkyl, -NO_2, or nonsubstituted or substituted phenyl, wherein the substituted phenyl can be substituted with one or more substituents selected from the group consisting of straight or branched C_1-C_3 alkyl, straight or branched C_1-C_3 alkoxy and -NO_2;
R^3 is hydrogen, -(C=O)OR^6 or nonsubstituted or substituted 5-7 membered heterocycloalkyl containing one or more heteroatoms selected from the group consisting of N, O and S, at this time, the substituted heterocycloalkyl can be substituted with one or more straight or branched C_1-C_3 alkyl groups;
A is -(C=O)NH-, -NH(C=O)-, -(C=O)O- or -O(C=O)-; and
m and n can independently be integers of 0-5.

[0022] Moreover, in formula 1 above,

R^1 and R^2 are independently methyl or nonsubstituted or substituted phenyl, wherein the substituted phenyl can be substituted with one or more substituents selected from the group consisting of methyl, methoxy and -NO_2;
R^3 is -(C=O)OH or piperidinyl substituted with one or more methyl groups;
A is -(C=O)NH- or -NH(C=O)-; and
m and n can independently be integers of 0-3.

[0023] The said pruritus can be histaminergic pruritus, non-histaminergic pruritus, pruritus induced by chloroquine, pruritus induced by dry skin or psoriatic pruritus (pruritus induced by psoriasis). The pruritus can also be induced by neurodermatitis, contact dermatitis, seborrheic dermatitis, autosensitized dermatitis, caterpillar dermatitis, sebum deficiency (asteatosis), senile pruritus skin, insect bites, photosensitive dermatitis, urticaria, prurigo, herpes, impetigo, eczema, tinea, lichen, scabies or acne vulgaris. At this time, in the case of histaminergic pruritus, the pharmaceutical composition of the present invention can be effective in treating or preventing pruritus by blocking the activity of histamine via reversible/competitive antagonism against hH1R (human Histamine 1 Receptor).
[0024] The pharmaceutical composition of the present invention can also be effective in treating or preventing pruritus induced by chloroquine by blocking the activity of chloroquine via reversible/competitive antagonism against MRGPR X1.
[0025] The pharmaceutically acceptable salt of the compound of formula 1 can be prepared by the conventional method known to those in the art. For example, the pharmaceutically acceptable salt includes salts of inorganic acids such as hydrochloric acid, bromic acid, sulfuric acid, sodium hydrogen sulfate, phosphoric acid, nitric acid and carbonic acid; salts of organic acids such as formic acid, acetic acid, propionic acid, oxalic acid, succinic acid, benzoic acid, citric acid, maleic acid, malonic acid, tartaric acid, gluconic acid, lactic acid, gestyic acid, fumaric acid, lactobionic acid, salicylic acid and acetylsalicylic acid (aspirin); salts of amino acids such as glycine, alanine, vanillin, isoleucine, serine, cysteine, cystine, aspartic acid, glutamine, lysine, arginine, tyrosine and proline; salts of sulfonic acids such as methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid and toluenesulfonic acid; metal salts generated by reaction with alkali metals such as sodium and potassium; or ammonium ion salts.
[0026] The acid addition salt can be prepared by the conventional method known to those in the art. For example, the derivative represented by formula 1 is dissolved in an organic solvent such as methanol, ethanol, acetone, methylenechloride and acetonitrile, to which organic acid or inorganic acid is added to induce precipitation. Then, the precipitate is filtered and dried to give the salt. Or the solvent and the excessive acid are distillated under reduced pressure, followed by drying and crystallization in an organic solvent to give the salt.
[0027] In addition, a pharmaceutically acceptable metal salt can be prepared by using a base. Alkali metal or alkali earth metal salt is obtained by the following processes: dissolving the compound in excessive alkali metal hydroxide or alkali earth metal hydroxide solution; filtering non-soluble compound salt; evaporating the remaining solution and drying thereof. At this time, the metal salt is preferably prepared in the pharmaceutically suitable form of sodium, potassium, or calcium salt. And the corresponding silver salt is prepared by the reaction of alkali metal or alkali earth metal salt with proper silver salt (ex; silver nitrate).
[0028] Furthermore, the compound represented by formula 1 can be used in the form of solvates, optical isomers,

hydrates, etc., which may be prepared therefrom, as well as the pharmaceutically acceptable salts thereof.

[0029] The pharmaceutical composition of the present invention can be formulated by adding non-toxic and pharmaceutically acceptable carriers, adjuvants and excipients according to the conventional methods. For example, the pharmaceutical composition of the present invention can be formulated for oral or parenteral administration in the forms of tablets, capsules, troches, solutions and suspensions, etc.

[0030] The compound represented by formula 1 or the pharmaceutically acceptable salt thereof can be administered in various oral and parenteral formulations during clinical administration. When the compound represented by formula 1 or the pharmaceutically acceptable salt thereof is formulated, generally used diluents or excipients such as fillers, extenders, binders, wetting agents, disintegrating agents and surfactants are used. Solid formulations for oral administration are tablets, pills, powders, granules and capsules. These solid formulations are prepared by mixing one or more compounds with one or more suitable excipients such as starch, calcium carbonate, sucrose or lactose, gelatin, etc. Except for the simple excipients, lubricants, for example magnesium stearate, talc, etc, can be used. Liquid formulations for oral administrations are suspensions, solutions, emulsions and syrups, and the above-mentioned formulations can contain various excipients such as wetting agents, sweeteners, aromatics and preservatives in addition to generally used simple diluents such as water and liquid paraffin. Formulations for parenteral administration are sterilized aqueous solutions, water-insoluble excipients, suspensions and emulsions. Water insoluble excipients and suspensions can contain, in addition to the active compound or compounds, propylene glycol, polyethylene glycol, vegetable oil like olive oil, injectable ester like ethylolate, etc.

[0031] The pharmaceutical composition comprising the compound represented by formula 1 or the pharmaceutically acceptable salt thereof as an active ingredient can be administered by parenterally and the parenteral administration includes subcutaneous injection, intravenous injection, intramuscular injection, or intrathoracic injection.

[0032] To prepare the compound represented by formula 1 or the pharmaceutically acceptable salt thereof as a formulation for parenteral administration, the compound represented by formula 1 or the pharmaceutically acceptable salt thereof is mixed with a stabilizer or a buffering agent in water to produce a solution or a suspension, which is then formulated as ampoules or vials. The composition herein can be sterilized and additionally contains preservatives, stabilizers, wettable powders or emulsifiers, salts and/or buffers for the regulation of osmotic pressure, and other therapeutically useful materials, and the composition can be formulated by the conventional mixing, granulating or coating method.

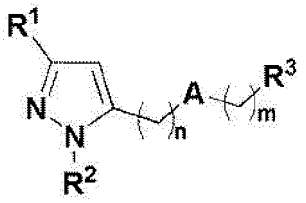
[0033] The formulations for oral administration are exemplified by tablets, pills, hard/soft capsules, solutions, suspensions, emulsions, syrups, granules, elixirs, and troches, etc. These formulations can include diluents (for example, lactose, dextrose, sucrose, mannitol, sorbitol, cellulose, and/or glycine) and lubricants (for example, silica, talc, stearate and its magnesium or calcium salt, and/or polyethylene glycol) in addition to the active ingredient. Tablets can include binding agents such as magnesium aluminum silicate, starch paste, gelatine, methylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrolidone, and if necessary disintegrating agents such as starch, agarose, alginic acid or its sodium salt or azeotropic mixtures and/or absorbents, coloring agents, flavours, and sweeteners can be additionally included thereto.

[0034] In addition, the excipients that can be used in the pharmaceutical composition according to the present invention include sweeteners, binders, solubilizers, dissolution aids, wetting agents, emulsifiers, isotonic agents, adsorbents, disintegrants, antioxidants, preservatives, lubricants, fillers, fragrances, etc. For example, lactose, dextrose, sucrose, mannitol, sorbitol, cellulose, glycine, silica, talc, stearic acid, sterin, magnesium stearate, magnesium aluminum silicate, starch, gelatin, tragacanth rubber, alginic acid, sodium alginate, methyl cellulose, sodium carboxylmethyl cellulose, agar, water, ethanol, polyethylene glycol, polyvinylpyrrolidone, sodium chloride, calcium chloride, orange essence, strawberry essence, vanilla flavor and the like can be used as the excipients.

[0035] The effective dosage of the pharmaceutical composition of the present invention can be determined according to age, weight, gender, administration method, health condition, and severity of disease. The dosage is generally 0.01 ~ 5000 mg/day based on an adult patient weighing 70 kg, which can be administered once or several times a day at intervals of a certain time depending on the judgment of a doctor or a pharmacist.

[0036] The present invention also provides a health functional food composition comprising a compound represented by formula 1 below or a pharmaceutically acceptable salt thereof as an active ingredient for preventing or alleviating pruritus.

[Formula 1]



[0037] In formula 1 above,

R^1 and R^2 are independently straight or branched C_1-C_6 alkyl, straight or branched C_1-C_6 alkoxy, -NO_2,-NR^4R^5 or nonsubstituted or substituted C_6-C_{12} aryl, wherein the substituted C_6-C_{12} aryl can be substituted with one or more substituents selected from the group consisting of halogen, straight or branched C_1-C_6 alkyl, straight or branched C_1-C_6 alkoxy, -NO_2 and-NR^4R^5, at this time, R^4 and R^5 are independently hydrogen or straight or branched C_1-C_6 alkyl;
R^3 is hydrogen, -(C=O)OR^6 or nonsubstituted or substituted 3-10 membered heterocycloalkyl or heterocycloalkenyl containing one or more heteroatoms selected from the group consisting of N, O and S, at this time, R^6 can be hydrogen or straight or branched C_1-C_6 alkyl, wherein the substituted heterocycloalkyl or heterocycloalkenyl can be substituted with one or more substituents selected from the group consisting of halogen, straight or branched C_1-C_6 alkyl and straight or branched C_1-C_6 alkoxy;
A is -NH-, -O-, -S-, -(C=O)NH-, -NH(C=O)-,-(C=O)O- or -O(C=O)-; and
m and n can independently be integers of 0-8.

[0038] In addition, in formula 1 above,

R^1 and R^2 are independently straight or branched C_1-C_3 alkyl, straight or branched C_1-C_3 alkoxy, -NO_2,-NR^4R^5 or nonsubstituted or substituted C_6-C_{10} aryl, wherein the substituted C_6-C_{10} aryl can be substituted with one or more substituents selected from the group consisting of halogen, straight or branched C_1-C_3 alkyl, straight or branched C_1-C_3 alkoxy, -NO_2 and-NR^4R^5, at this time, R^4 and R^5 are independently hydrogen or straight or branched C_1-C_3 alkyl;
R^3 is hydrogen, -(C=O)OR^6 or nonsubstituted or substituted 3-8 membered heterocycloalkyl or heterocycloalkenyl containing one or more heteroatoms selected from the group consisting of N, O and S, at this time, R^6 can be hydrogen or straight or branched C_1-C_3 alkyl, wherein the substituted heterocycloalkyl or heterocycloalkenyl can be substituted with one or more substituents selected from the group consisting of halogen, straight or branched C_1-C_3 alkyl and straight or branched C_1-C_3 alkoxy;
A is -NH-, -O-, -S-, -(C=O)NH-, -NH(C=O)-, - (C=O)O- or -O(C=O)-; and
m and n can independently be integers of 0-6.

[0039] Further, in formula 1 above,

R^1 and R^2 are independently straight or branched C_1-C_3 alkyl, -NO_2, or nonsubstituted or substituted phenyl, wherein the substituted phenyl can be substituted with one or more substituents selected from the group consisting of straight or branched C_1-C_3 alkyl, straight or branched C_1-C_3 alkoxy and -NO_2;
R^3 is hydrogen, -(C=O)OR^6 or nonsubstituted or substituted 5-7 membered heterocycloalkyl containing one or more heteroatoms selected from the group consisting of N, O and S, at this time, the substituted heterocycloalkyl can be substituted with one or more straight or branched C_1-C_3 alkyl groups;
A is -(C=O)NH-, -NH(C=O)-, -(C=O)O- or -O(C=O)-; and
m and n can independently be integers of 0-5.

[0040] Moreover, in formula 1 above,

R^1 and R^2 are independently methyl or nonsubstituted or substituted phenyl, wherein the substituted phenyl can be substituted with one or more substituents selected from the group consisting of methyl, methoxy and -NO_2;
R^3 is -(C=O)OH or piperidinyl substituted with one or more methyl groups;
A is -(C=O)NH- or -NH(C=O)-; and
m and n can independently be integers of 0-3.

[0041] The health functional food composition according to the present invention can be prepared by adding the

compound of formula 1 above or the pharmaceutically acceptable salt thereof to food or beverages for the purpose of preventing or alleviating pruritus.

[0042] The food herein is not limited. For example, the composition of the present invention can be added to drinks, meats, sausages, breads, biscuits, rice cakes, chocolates, candies, snacks, pizza, ramyuns, flour products, gums, dairy products including ice cream, soups, beverages, alcohol drinks and vitamin complex, etc, and in wide sense, almost every health functional food can be included.

[0043] The compound represented by formula 1 of the present invention can be used as food additive. In that case, the compound can be added as it is or as mixed with other food components according to the conventional method. The mixing ratio of active ingredients can be regulated according to the purpose of use (prevention or alleviation). In general, the compound represented by formula 1 of the present invention can be added at 0.1 to 90 weight parts by the total food weight. However, if long term administration is required for health and hygiene or regulating health condition, the content can be lower than the above but higher content can be accepted as well since the compound has been proved to be very safe.

[0044] The composition for health beverages of the present invention can additionally include various flavors or natural carbohydrates, etc, like other beverages in addition to the compound. The natural carbohydrates above can be one of monosaccharides such as glucose and fructose; disaccharides such as maltose and sucrose; polysaccharides such as dextrin and cyclodextrin; and sugar alcohols such as xilytole, sorbitol and erythritol. Besides, natural sweetening agents (thaumatin, stevia extract, for example rebaudioside A, glycyrrhizin, etc.) and synthetic sweetening agents (saccharin, aspartame, etc.) can be included as a sweetening agent. The content of the natural carbohydrate is preferably 1-20 g and more preferably 5-12 g in 100 g of the composition of the present invention.

[0045] In addition to the ingredients mentioned above, the compound represented by formula 1 of the present invention can include in variety of nutrients, vitamins, minerals (electrolytes), flavors including natural flavors and synthetic flavors, coloring agents and extenders (cheese, chocolate, etc.), pectic acid and its salts, alginic acid and its salts, organic acid, protective colloidal viscosifiers, pH regulators, stabilizers, antiseptics, glycerin, alcohols, carbonators which used to be added to soda, etc. The pyrazole derivative represented by formula 1 of the present invention can also include natural fruit juice, fruit beverages and fruit flesh addable to vegetable beverages. All the mentioned ingredients can be added singly or together. The mixing ratio of those ingredients does not matter in fact, but in general, each can be added by 0.1-20 weight part per 100 weight part of the pyrazole derivative represented by formula 1 of the present invention.

[0046] Further, the present invention provides a screening method of a compound for preventing or treating pruritus, which comprises the following steps:

treating a pruritus-inducing substance to cells expressing MRGPR X1 (Mas-related G protein-coupled receptor), and culturing the cells (step 1); and

treating a candidate material to the pruritus-induced cells, and measuring the inhibition of MRGPR X1 activity (step 2).

[0047] At this time, the pruritus-inducing substance of step 1 can be chloroquine, and any histamine-independent pruritus-inducing substance which does not mediated by histamine can be used without limitation. On the other hand, the pruritus-inducing substance can be an acute pruritus-inducing substance.

[0048] In addition, the present invention provides a screening method of an active material for preventing or treating pruritus, which comprises the following steps:

treating a pruritus-inducing substance to cells expressing hH1R (human Histamine 1 Receptor), and culturing the cells (step 1); and

treating a candidate material to the pruritus-induced cells, and measuring the inhibition of hH1R activity (step 2).

[0049] At this time, the pruritus-inducing substance of step 1 can be one or more substances selected from the group consisting of histamine, interleukin-1, cytokine, serotonin, acethylcholine, substance P, leukotrine and prostaglandin, and any histamine-dependent pruritus-inducing substance can be used without limitation.

[0050] In the screening method according to the present invention, the measurement of step 2 can be performed by one or more methods selected from the group consisting of fluorescence assay, fluorescence resonance energy transfer assay, bioluminescence resonance energy transfer assay, fluorescence polarization assay, Western blotting, immunoprecipitation assay, dual luciferasereporter assay, enzyme-linked immunosorbent assay(ELISA) and immunohistochemistry, and any measurement method commonly used in the art can be used without limitation.

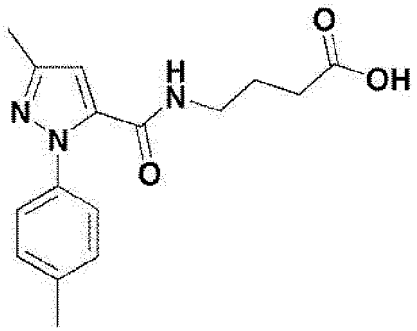
[0051] Practical and presently preferred embodiments of the present invention are illustrative as shown in the following Examples.

[0052] However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

<Example 1> Pharmaceutical composition for preventing or treating pruritus

[0053] A pharmaceutical composition comprising the pyrazole derivative represented by formula 2 for preventing or treating pruritus was prepared in Experimental Example 1. At this time, the pyrazole derivative represented by formula 2 was purchased from Com Genex (CAS Number. 1023449-28-6).

[Formula 2]



<Experimental Example 1> Screening of active material for preventing or treating pruritus

[0054] The following experiment was performed using the screening method of the present invention in order to find a compound suitable for the prevention or treatment of pruritus.

1. MRGPR X1 stable cell line 제조

1. Preparation of MRGPR X1 stable cell line

[0055] Human MRGPRX1 gene was sub-cloned in pcDNA5 FRT vector, which was transfected into HEK 293T FRT cells. The cells were cultured in a 37°C, 5% CO_2 incubator for 48 hours.

[0056] To confirm the amount with which a single colony is well formed, the transfected cells were serially diluted and then transferred and distributed on 100 mm dish evenly. The medium was replaced with a fresh medium containing 50 μg/mℓ of hygromycin every 3-4 days. The cells were cultured until colonies were formed.

[0057] Once colonies were formed, only a single colony was transferred to a 24 well plate, followed by culture. Then, well grown cells were transferred to a 6 well plate, followed by culture. Antibiotic selection was performed. To select those cells which were not able to survive in Zeocin medium but were well grown in hygromycin medium, the cells were seeded in 6 well plates. A medium containing 50 μg/mℓ of Zeocin was added to one plate and a medium containing 50 μg/mℓ of hygromycin was added to the other plate. The cells that died in Zeocin medium but grew well in hygromycin medium were selected, with which PCR and β-galactosidase assay were performed to confirm the success of transfection.

2. MRGPR X1 screening assay

[0058] The following experiment was performed to find a material effective in pruritus using the Mrgpr X1 stable cell line prepared above.

[0059] First, MRGPR X1 stable cells were counted to make 8000 cells/well, followed by seeding in a Poly-D-Lysine coated plate 384 well black/clear plate. Then, the cells were cultured in a 37°C, 5% CO_2 incubator for overnight.

[0060] The subsequent procedure was performed using Biomex FX (Beckman). After eliminating the medium, flo-3 AM dye dissolved in Na-HEPES buffer (5 mM KCl, 2 mM $MgCl_2$, 140 mM NaCl, 10 mM NaOH-HEPES, pH7.2) at the concentration of 2.5 μM was added to the plate (25 $\mu\ell$/well), followed by culture in a 37°C, 5% CO_2 incubator for 30 minutes.

[0061] During the culture, a compound plate was prepared. Particularly, a 6 mM original stock plate (96 plate form) was pre-mixed well and then an intermediate plate was prepared. Na-HEPES buffer containing 1 mM chloroquine dissolved therein was distributed in a 384 clear V bottom plate (49 $\mu\ell$/well) using Multidrop. Then, 1 $\mu\ell$ was taken from the original stock plate (6 mM, 100% DMSO), which was diluted in the intermediate plate (120 uM compound, 1 mM chloroquine, 2% DMSO), leading to the preparation of a final compound plate.

[0062] Na-HEPES buffer containing 1 mM chloroquine dissolved therein was distributed in the 384 clear plate (40

$\mu\ell$/well) using Multidrop, and 10 $\mu\ell$ was taken from the intermediate plate, which was diluted in the final compound plate (24 μM compound, 1 mM chloroquine, 0.4% DMSO).

[0063] Upon completion of the incubation, dye was discarded and Na-HEPES buffer was added thereto (25 $\mu\ell$/well) for washing, and then buffer was added thereto (25 $\mu\ell$/well) again. 25 $\mu\ell$ of the primary candidate material was added to each well of the assay plate containing 25 $\mu\ell$ of buffer, followed by reading the plate (final 12 μM compound, 500 μM chloroquine, 0.2% DMSO). Vehicle was treated by dissolving 500 μM chloroquine in 0.2% DMSO.

[0064] The experimental results were analyzed using a microplate reader (FlexstaionII^{384}, Molecular Devices). Fluorescence assay was performed (Ex 488nm, Em 535nm) at the intervals of 3.2 seconds for 80 seconds. As a result, the compounds with good test results were sorted, based on which, 910 secondary candidate materials for the treatment of pruritus were selected.

3. HumanHistamine1 Receptor antagonist assay

[0065] Human histamine 1 receptor antagonist assay was performed with those 910 secondary candidate materials selected in the MRGPR X1 experiment above.

[0066] HEK 293T cells were transiently transfected with 2 μg of human histamine 1 receptor, followed by seeding at the density of 4000 cells/well. The cells were cultured in a 37°C, 5% CO_2 incubator for 48 hours.

[0067] The subsequent procedure was performed using Biomex FX. After eliminating the medium, flo-3 AM dye dissolved in Na-HEPES buffer (5 mM KCl, 2 mM $MgCl_2$, 140 mM NaCl, 10 mM NaOH-HEPES, pH7.2) at the concentration of 2.5 μM was added to the plate (25 $\mu\ell$/well) of the vehicle and experimental groups, and flo-3 AM dye dissolved in Na-HEPES buffer containing 5 uM diphenhydramine dissolved therein at the concentration of 2.5 μM was added to the plate (25 $\mu\ell$/well) of the control group, followed by culture in a 37°C, 5% CO_2 incubator for 30 minutes.

[0068] During the culture, a compound plate was prepared. Particularly, a 6 mM original stock plate (96 plate form) was pre-mixed well and then an intermediate plate was prepared. Na-HEPES buffer containing 2 μM histamine dissolved therein was distributed in a 384 clear V bottom plate (49 $\mu\ell$/well) using Multidrop. Then, 1 $\mu\ell$ was taken from the original stock plate (6 mM, 100% DMSO), which was diluted in the intermediate plate (120 μM compound, 2 μM histamine, 2% DMSO), leading to the preparation of a final compound plate.

[0069] Na-HEPES buffer containing 2 μM histamine dissolved therein was distributed in the 384 clear plate (40 $\mu\ell$/well) using Multidrop, and 10 $\mu\ell$ was taken from the intermediate plate, which was diluted in the final compound plate (24 μM compound, 2 μM histamine, 0.4% DMSO, excluding control group wells).

[0070] Upon completion of the incubation, dye was discarded and Na-HEPES buffer was added thereto (25 $\mu\ell$/well) for washing, and then buffer was added thereto (25 $\mu\ell$/well) again (excluding control group wells). Na-HEPES buffer containing 5 μM diphenhydramine and 2 μM histamine dissolved therein was added to the control group wells (25 $\mu\ell$/well). Finally, 25 $\mu\ell$ of the compound was added to each well of the assay plate containing 25 $\mu\ell$ of buffer, followed by reading the plate (final 12 μM compound, 1 μM histamine, 0.2% DMSO).

[0071] The experimental results were analyzed using a microplate reader (FlexstaionII^{384}, Molecular Devices). Fluorescence assay was performed (Ex 488nm, Em 535nm) at the intervals of 3.2 seconds for 80 seconds. As a result, 25 compounds having excellent effects were selected from the 910 compounds selected above. A few kinds of scaffolds representing the similarity in their structures were identified. Among them, 4 kinds of compounds having the representative structure were derived. At last, compound #3 which was confirmed to be excellent in treating pruritus and at the same time having excellent solubility in blood was selected as a final therapeutic material for pruritus. Compound #3 is a compound represented by formula 2 of Example 1.

4. Analysis of experiment results

[0072] To evaluate the activity of Mrgpr X1 expressing cells and hH1R expressing cells according to compound #3, the final therapeutic material for pruritus, data collected from the experiments above were compared and analyzed.

[0073] Figure 1 is a graph showing the results of fluorescence analysis performed after treating Mrgpr X1 cells with chloroquine alone, and

Figure 2 is a graph showing the results of fluorescence analysis performed after treating Mrgpr X 1 cells with a pharmaceutical composition comprising the pyrazole derivative of Example 1 together with chloroquine.

[0074] As shown in Figure 1 and Figure 2, fluorescence analysis result was declined approximately 54% when the pharmaceutical composition of the present invention was co-treated with chloroquine, compared with when chloroquine was treated alone.

[0075] Therefore, the pharmaceutical composition for preventing or treating pruritus according to the present invention can be effectively used as a preventive or therapeutic agent for non-histaminergic pruritus since it can relieve the symptoms of pruritus by inhibiting the activity of intracellular Mrgpr X1.

[0076] Figure 3 is a graph showing the results of fluorescence analysis performed after treating hH1R cells with

histamine alone,
Figure 4 is a graph showing the results of fluorescence analysis performed after treating hH1R cells with histamine and diphenhydramine, and
Figure 5 is a graph showing the results of fluorescence analysis performed after treating hH1R cells with a pharmaceutical composition comprising the pyrazole derivative of Example 1 together with histamine.

[0077] As shown in Figures 3 ~ 5, the pharmaceutical composition of the present invention was able to reduce approximately 94%, considering the inhibition effect when histamine and diphenhydramine (H1 receptor antagonist) were treated together as 100%.

[0078] Therefore, the pharmaceutical composition for preventing or treating pruritus according to the present invention can be effectively used as a preventive or therapeutic agent for histaminergic pruritus since it can relieve the symptoms of pruritus by inhibiting the activity of intracellular hH1R.

<Experimental Example 2> Animal model experiment of the therapeutic agent for pruritus of the present invention

[0079] To confirm the *in vivo* treatment effect of the pharmaceutical composition for treating pruritus of the present invention, pruritus evaluation in the histamine mouse model, pruritus evaluation in the chloroquine mouse model, pruritus evaluation in the dry skin mouse model, pruritus evaluation in the psoriasis mouse model and mouse motor ability test were performed.

1. Pruritus evaluation in histamine mouse model

[0080] An experiment was performed as follows in order to evaluate the pruritus treatment effect of the pharmaceutical composition of Example 1 in the histamine mouse model.

[0081] Seven week-old C57BL6 mice were placed in a new cage and adapted for 30 minutes before starting the experiment. As for the control group, 3% DMSO was dissolved in physiological saline (0.9% saline), which was intraperitoneally injected (50 $\mu\ell$/mouse). The animal model mice were intraperitoneally injected with the pharmaceutical composition of Example 1 (30 mg/kg) dissolved in physiological saline (0.9% saline) containing 3% DMSO (50 $\mu\ell$/mouse). The mice were also placed in the same cage above for 30 minutes.

[0082] Then, control and animal model mice were subcutaneously injected with 500 μg/50 $\mu\ell$ of histamine on the dorsal side, and their behaviors were recorded with a digital video camera for 30 minutes. The number of scratches was recorded while playing back the recorded video file on a computer. A continuous action of scratching the skin from when the mouse lifted its hind foot off the floor and to when the mouse put it back on the floor was considered 1 scratch, and the number of scratches counted for 30 minutes was used as index for evaluating pruritus.

[0083] Figure 6 is a graph illustrating the evaluation of pruritus treatment effect when the histamine mouse model is treated with a pharmaceutical composition comprising the pyrazole derivative of Example 1.

[0084] As shown in Figure 6, the average number of total scratches accumulated for 30 minutes in the control group was 118.38 times, and the average number of scratches in the experimental group administered with the pharmaceutical composition of the present invention was 39.90 times.

[0085] Therefore, the pharmaceutical composition comprising the pyrazole derivative according to the present invention can be effectively used as a pharmaceutical composition for treating or preventing pruritus since it showed an effect of reducing histaminergic pruritus.

2. Pruritus evaluation in chloroquine mouse model

[0086] An experiment was performed as follows in order to evaluate the pruritus treatment effect of the pharmaceutical composition of Example 1 in the chloroquine mouse model.

[0087] Seven week-old C57BL6 mice were placed in a new cage and adapted for 30 minutes before starting the experiment. As for the control group, 3% DMSO was dissolved in physiological saline (0.9% saline), which was intraperitoneally injected (50 $\mu\ell$/mouse). The animal model mice were intraperitoneally injected with the pharmaceutical composition of Example 1 (30 mg/kg) dissolved in physiological saline (0.9% saline) containing 3% DMSO (50 $\mu\ell$/mouse). The mice were also placed in the same cage above for 30 minutes.

[0088] Then, control and animal model mice were subcutaneously injected with 200 μg/50 $\mu\ell$ of chloroquine on the dorsal side, and their behaviors were recorded with a digital video camera for 30 minutes. The number of scratches was recorded while playing back the recorded video file on a computer. A continuous action of scratching the skin from when the mouse lifted its hind foot off the floor and to when the mouse put it back on the floor was considered 1 scratch, and the number of scratches counted for 30 minutes was used as index for evaluating pruritus.

[0089] Figure 7 is a graph illustrating the evaluation of pruritus treatment effect when the chloroquine mouse model is treated with a pharmaceutical composition comprising the pyrazole derivative of Example 1.

[0090] As shown in Figure 7, the average number of total scratches accumulated for 30 minutes in the control group was 425.00 times, and the average number of scratches in the experimental group administered with the pharmaceutical composition of the present invention was 150.50 times.

[0091] Therefore, it was confirmed from the above results that the pharmaceutical composition comprising the pyrazole derivative compound according to the present invention was significantly effective in not only alleviating histaminergic pruritus but also alleviating non-histaminergic pruritus, suggesting that the composition of the present invention can be effectively used for the treatment of non-histaminergic pruritus.

3. Pruritus evaluation in dry skin mouse model

[0092] An experiment was performed as follows in order to evaluate the pruritus treatment effect of the pharmaceutical composition of Example 1 in the dry skin mouse model.

[0093] First, a dry skin mouse model was constructed. A dry skin mouse model was prepared according to the instruction described Jpn J Pharmacol. 2002 Mar;88(3):285-92. ENTOBAR® was mixed with physiological saline (0.9% saline) at the ratio of 1:1, which was intraperitoneally injected in 7-week-old C57BL6 mice (20 $\mu\ell$/mouse) to anesthetize the mice. Then, the hair of the right nape was shaved using a clipper. Ethyl ether and acetone were mixed at the ratio of 1:1. The mixture was soaked in absorbent cotton and the right nape was rubbed with the absorbent cotton for 1 minute. Then, absorbent cotton soaked with water was used to rub in the same way. This process was performed once in the morning and once in the afternoon for 5 days.

[0094] Next, pruritus evaluation in the dry skin mouse model was performed. In the afternoon on Day 5, the control group mice were intraperitoneally injected with 3% DMSO dissolved in physiological saline (0.9% saline) (50 $\mu\ell$/mouse). The animal model mice were intraperitoneally injected with the pharmaceutical composition of Example 1 (30 mg/kg) dissolved in physiological saline (0.9% saline) containing 3% DMSO (50 $\mu\ell$/mouse). The mice were also placed in the same cage for 30 minutes. 30 minutes later, their behaviors were recorded with a digital camera for 30 minutes. The number of scratches was recorded while playing back the recorded video file on a computer. A continuous action of scratching the skin from when the mouse lifted its hind foot off the floor and to when the mouse put it back on the floor was considered 1 scratch, and the number of scratches counted for 30 minutes was used as index for evaluating pruritus.

[0095] Figure 8 is a graph illustrating the evaluation of pruritus treatment effect when the dry skin mouse model is treated with a pharmaceutical composition comprising the pyrazole derivative of Example 1.

[0096] As shown in Figure 8, the average number of total scratches accumulated for 30 minutes in the control group was 130.00 times, and the average number of total scratches in the experimental group administered with the pharmaceutical composition of the present invention was 14.00 times.

[0097] Therefore, it was confirmed from the above results that the pharmaceutical composition comprising the pyrazole derivative compound according to the present invention was significantly effective in alleviating pruritus induced by dry skin, suggesting that the composition of the present invention can be effectively used for the treatment of pruritus induced by dry skin or dry skin.

4. Evaluation of Rota rod motor condition

[0098] To evaluate the *in vivo* stability of the pharmaceutical composition according to the present invention, Rota rod motion condition test was performed with a mouse model.

[0099] The test mouse was placed on the Rota rod device and then the speed was raised from 4 to 40 until the mouse fell. When the mouse fell, it was moved to a cage. The procedure was repeated three times a day for 3 consecutive days to adapt the mouse to the Rota rod device. After three days of adaptation, the mouse was moved to a cage where it was stabilized.

[0100] As for the control group, 3% DMSO was dissolved in physiological saline (0.9% saline), which was intraperitoneally injected (50 $\mu\ell$/mouse). The animal model mice were intraperitoneally injected with the pharmaceutical composition of Example 1 (30 mg/kg) dissolved in physiological saline (0.9% saline) containing 3% DMSO (50 $\mu\ell$/mouse). The mice were also placed in the same cage above for 30 minutes. The experiment above was repeated three times like the adaptation test above, and the average time of falling was calculated.

[0101] Figure 9 is a graph showing the results of Rota rod motion condition test with the mouse treated with a pharmaceutical composition comprising the pyrazole derivative of Example 1.

[0102] As shown in Figure 9, when comparing the motor condition ability between the control group and the experimental group administered with the pharmaceutical composition of the present invention, there was no significant difference between the groups.

[0103] Therefore, the pharmaceutical composition containing the pyrazole derivative according to the present invention was confirmed not to cause any adverse reaction *in vivo* when administered, but to maintain a stable activity, so that the composition can be effectively used as a pharmaceutical composition for preventing or treating pruritus.

5. Pruritus evaluation in psoriatic mouse model

[0104] An experiment was performed as follows in order to evaluate the pruritus treatment effect of the pharmaceutical composition of Example 1 in the psoriatic mouse model.

[0105] First, a psoriatic mouse model was constructed. A psoriatic mouse model was prepared according to the instruction described Pain. 2016 Nov; 2536-2543. Zoletil (15 mg/kg) was mixed with saline at the ratio of 1:1 and then mixed with Rompun (5 mg/kg), which was intraperitoneally injected in 7-week-old C57BL6 mice to anesthetize the mice. The mouse back was primarily depilated in the size of 2.5 x 2 cm using a clipper, and then secondly depilated using a straight blade. Vaseline cream was rubbed on the depilated area of the normal group for 7 days using a cotton swap. Aldara cream (62.5 mg, 5% imiquimod) was rubbed on the depilated area of the control group and the experimental group for 7 days using a cotton swap.

[0106] Next, pruritus evaluation in the psoriatic mouse model was performed. On day 8, the normal group and control group mice were intraperitoneally injected with 3% DMSO dissolved in physiological saline (0.9% saline) (50 $\mu\ell$/mouse). The experimental group mice were intraperitoneally injected with the pharmaceutical composition of Example 1 (30 mg/kg) dissolved in physiological saline (0.9% saline) containing 3% DMSO (50 $\mu\ell$/mouse). The mice were placed in the same cage for 30 minutes. 30 minutes later, their behaviors were recorded with a digital camera for 30 minutes. The number of scratches was recorded while playing back the recorded video file on a computer. A continuous action of scratching the skin from when the mouse lifted its hind foot off the floor and to when the mouse put it back on the floor was considered 1 scratch, and the number of scratches counted for 30 minutes was used as index for evaluating pruritus.

[0107] Figure 10 is a graph illustrating the evaluation of pruritus treatment effect when the psoriatic mouse model is treated with a pharmaceutical composition comprising the pyrazole derivative of Example 1.

[0108] As shown in Figure 10, the average number of total scratches accumulated in the normal group was 20.00 times, and the average number of total scratches in the control group (Aldara cream treated group) was 71.00 times. In the group administered with the pharmaceutical composition containing the pyrazole derivative according to the present invention, the average number of total scratches was 1.00, indicating the scratch times were significantly reduced.

[0109] Therefore, it was confirmed from the above results that the pharmaceutical composition comprising the pyrazole derivative according to the present invention was significantly effective in alleviating pruritus induced by psoriasis, suggesting that the composition of the present invention can be effectively used for the treatment of pruritus induced by psoriasis or psoriasis.

<Manufacturing Example 1> Preparation of powders

Compound represented by formula 1	2 g
Lactose	1 g

[0110] Powders were prepared by mixing all the above components, which were filled in airtight packs according to the conventional method for preparing powders.

<Manufacturing Example 2> preparation of tablets

Compound represented by formula 1	100 mg
Corn starch	100 mg
Lactose	100 mg
Magnesium stearate	2 mg

[0111] Tablets were prepared by mixing all the above components by the conventional method for preparing tablets.

<Manufacturing Example 3> Preparation of capsules

Compound represented by formula 1	100 mg
Corn starch	100 mg
Lactose	100 mg
Magnesium stearate	2 mg

[0112] Capsules were prepared by mixing all the above components, which were filled in gelatin capsules according to the conventional method for preparing capsules.

<Manufacturing Example 4> Preparation of injectable solutions

Compound represented by formula 1	100 mg
Mannitol	180 mg
$Na_2HPO_4 \cdot 2H_2O$	26 mg
Distilled water	2974 mg

[0113] Injectable solutions were prepared by containing all the above components in the amounts indicated according to the conventional method for preparing injectable solutions.

<Manufacturing Example 5> Preparation of ointments

Compound represented by formula 1	5 g
Cetyl palmitate	20 g
Cetanol	40 g
Stearyl alcohol	40 g
Myristan isopropyl	80 g
Polysorbate	60 g
Propyl p-hydroxybenzoate	1 g
Methyl p-hydroxybenzoate	1 g

[0114] Phosphoric acid and purified water proper amount

[0115] Ointments were prepared by containing all the above components in the amounts indicated according to the conventional method for preparing ointments.

<Manufacturing Example 6> Preparation of health functional foods

Compound represented by formula 1	500 ng
Vitamin complex	proper amount
Vitamin A acetate	70 mg
Vitamin E	1.0 mg
Vitamin	0.13 mg
Vitamin B2	0.15 mg
Vitamin B6	0.5 mg
Vitamin B12	0.2 mg
Vitamin C	10 mg
Biotin	10 mg
Nicotinamide	1.7 mg
Folic acid	50 mg
Calcium pantothenate	0.5 mg
Minerals	proper amount
Ferrous sulfate	1.75 mg
Zinc oxide	0.82 mg
Magnesium carbonate	25.3 mg
Potassium phosphate	15 mg
Calcium phosphate, dibasic	55 mg
Potassium citrate	90 mg
Calcium carbonate	100 mg
Magnesium chloride	24.8 mg

[0116] The vitamins and minerals appropriate for health functional foods were mixed according to the preferred mixing ratio but the composition ratio can be adjusted arbitrarily. After mixing the above components according to the conventional method for preparing health functional foods, granules were prepared and the granules were used for the preparation of health functional foods according to the conventional method.

<Manufacturing Example 7> Preparation of health beverages

Compound represented by formula 1	500 ng
Citric acid	1000 mg
Oligosaccharide	100 g
Maesil (Prunus mume) Extract	2 g
Taurine	1 g
Purified water	up to 900 mℓ

[0117] The above constituents were mixed according to the conventional method for preparing health beverages. The mixture was heated at 85°C for 1 hour with stirring and then filtered. The filtrate was loaded in 2 liter sterilized containers, which were sealed and sterilized again, stored in a refrigerator until they would be used for the preparation of a composition for health beverages.

[0118] The constituents appropriate for favorite beverages were mixed according to the preferred mixing ratio but the composition ratio can be adjusted according to regional and ethnic preferences such as demand class, demand country, and purpose of use, etc.

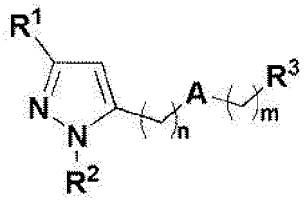
INDUSTRIAL APPLICABILITY

[0119] The pharmaceutical composition for preventing or treating pruritus according to the present invention can be effectively used as a preventive or therapeutic agent for non-histaminergic pruritus, a preventive or therapeutic agent for histaminergic pruritus, a therapeutic agent for dry skin and a therapeutic agent for psoriasis.

Claims

1. A pharmaceutical composition for preventing or treating pruritus comprising a compound represented by formula 1 below or a pharmaceutically acceptable salt thereof as an active ingredient:

[Formula 1]



(In formula 1,

R^1 and R^2 are independently straight or branched C_1-C_6 alkyl, straight or branched C_1-C_6 alkoxy, $-NO_2$, $-NR^4R^5$ or nonsubstituted or substituted C_6-C_{12} aryl, wherein the substituted C_6-C_{12} aryl can be substituted with one or more substituents selected from the group consisting of halogen, straight or branched C_1-C_6 alkyl, straight or branched C_1-C_6 alkoxy, $-NO_2$ and $-NR^4R^5$, at this time, R^4 and R^5 are independently hydrogen or straight or branched C_1-C_6 alkyl;
R^3 is hydrogen, $-(C=O)OR^6$ or nonsubstituted or substituted 3-10 membered heterocycloalkyl or heterocycloalkenyl containing one or more heteroatoms selected from the group consisting of N, O and S, at this time, R^6 can be hydrogen or straight or branched C_1-C_6 alkyl, wherein the substituted heterocycloalkyl or heterocycloalkenyl can be substituted with one or more substituents selected from the group consisting of halogen, straight or branched C_1-C_6 alkyl and straight or branched C_1-C_6 alkoxy;
A is -NH-, -O-, -S-, -(C=O)NH-, -NH(C=O)-,-(C=0)0- or -O(C=O)-; and
m and n can independently be integers of 0-8).

2. The pharmaceutical composition for preventing or treating pruritus according to claim 1, wherein:

R^1 and R^2 are independently straight or branched C_1-C_3 alkyl, straight or branched C_1-C_3 alkoxy, $-NO_2$,$-NR^4R^5$

or nonsubstituted or substituted C_6-C_{10} aryl, wherein the substituted C_6-C_{10} aryl can be substituted with one or more substituents selected from the group consisting of halogen, straight or branched C_1-C_3 alkyl, straight or branched C_1-C_3 alkoxy, $-NO_2$ and-NR^4R^5, at this time, R^4 and R^5 are independently hydrogen or straight or branched C_1-C_3 alkyl;
R^3 is hydrogen, -(C=O)OR^6 or nonsubstituted or substituted 3-8 membered heterocycloalkyl or heterocycloalkenyl containing one or more heteroatoms selected from the group consisting of N, O and S, at this time, R^6 can be hydrogen or straight or branched C_1-C_3 alkyl, wherein the substituted heterocycloalkyl or heterocycloalkenyl can be substituted with one or more substituents selected from the group consisting of halogen, straight or branched C_1-C_3 alkyl and straight or branched C_1-C_3 alkoxy;
A is -NH-, -O-, -S-, -(C=O)NH-, -NH(C=O)-,-(C=0)0- or -O(C=O)-; and
m and n can independently be integers of 0-6.

3. The pharmaceutical composition for preventing or treating pruritus according to claim 1, wherein:

R^1 and R^2 are independently straight or branched C_1-C_3 alkyl, $-NO_2$, or nonsubstituted or substituted phenyl, wherein the substituted phenyl can be substituted with one or more substituents selected from the group consisting of straight or branched C_1-C_3 alkyl, straight or branched C_1-C_3 alkoxy and $-NO_2$;
R^3 is hydrogen, -(C=O)OR^6 or nonsubstituted or substituted 5-7 membered heterocycloalkyl containing one or more heteroatoms selected from the group consisting of N, O and S, at this time, the substituted heterocycloalkyl can be substituted with one or more straight or branched C_1-C_3 alkyl groups;
A is -(C=O)NH-, -NH(C=O)-, -(C=0)0- or -O(C=O)-; and
m and n can independently be integers of 0-5.

4. The pharmaceutical composition for preventing or treating pruritus according to claim 1, wherein:

R^1 and R^2 are independently methyl or nonsubstituted or substituted phenyl, wherein the substituted phenyl can be substituted with one or more substituents selected from the group consisting of methyl, methoxy and $-NO_2$;
R^3 is -(C=O)OH or piperidinyl substituted with one or more methyl groups;
A is -(C=O)NH- or -NH(C=O)-; and
m and n can independently be integers of 0-3.

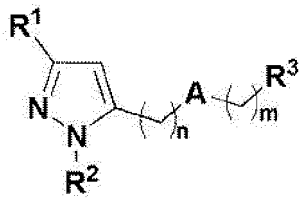
5. The pharmaceutical composition for preventing or treating pruritus according to claim 1, wherein the pruritus is histaminergic pruritus.

6. The pharmaceutical composition for preventing or treating pruritus according to claim 1, wherein the pruritus is non-histaminergic pruritus.

7. The pharmaceutical composition for preventing or treating pruritus according to claim 1, wherein the pruritus is a pruritus induced by one or more diseases selected from the group consisting of psoriasis, dry skin, neurodermatitis, contact dermatitis, seborrheic dermatitis, autosensitized dermatitis, caterpillar dermatitis, sebum deficiency (asteatosis), senile pruritus skin, insect bites, photosensitive dermatitis, urticaria, prurigo, herpes, impetigo, eczema, tinea, lichen, scabies and acne vulgaris.

8. A health functional food composition for preventing or alleviating pruritus comprising a compound represented by formula 1 below or a pharmaceutically acceptable salt thereof as an active ingredient:

[Formula 1]



(In formula 1,

R^1 and R^2 are independently straight or branched C_1-C_6 alkyl, straight or branched C_1-C_6 alkoxy, -NO_2,-NR^4R^5 or nonsubstituted or substituted C_6-C_{12} aryl, wherein the substituted C_6-C_{12} aryl can be substituted with one or more substituents selected from the group consisting of halogen, straight or branched C_1-C_6 alkyl, straight or branched C_1-C_6 alkoxy, -NO_2 and-NR^4R^5, at this time, R^4 and R^5 are independently hydrogen or straight or branched C_1-C_6 alkyl;
R^3 is hydrogen, -(C=O)OR^6 or nonsubstituted or substituted 3-10 membered heterocycloalkyl or heterocycloalkenyl containing one or more heteroatoms selected from the group consisting of N, O and S, at this time, R^6 can be hydrogen or straight or branched C_1-C_6 alkyl, wherein the substituted heterocycloalkyl or heterocycloalkenyl can be substituted with one or more substituents selected from the group consisting of halogen, straight or branched C_1-C_6 alkyl and straight or branched C_1-C_6 alkoxy;
A is -NH-, -O-, -S-, -(C=O)NH-, -NH(C=O)-,-(C=0)0- or -O(C=O)-; and
m and n can independently be integers of 0-8).

9. A screening method of a compound for preventing or treating pruritus, which comprises the following steps:

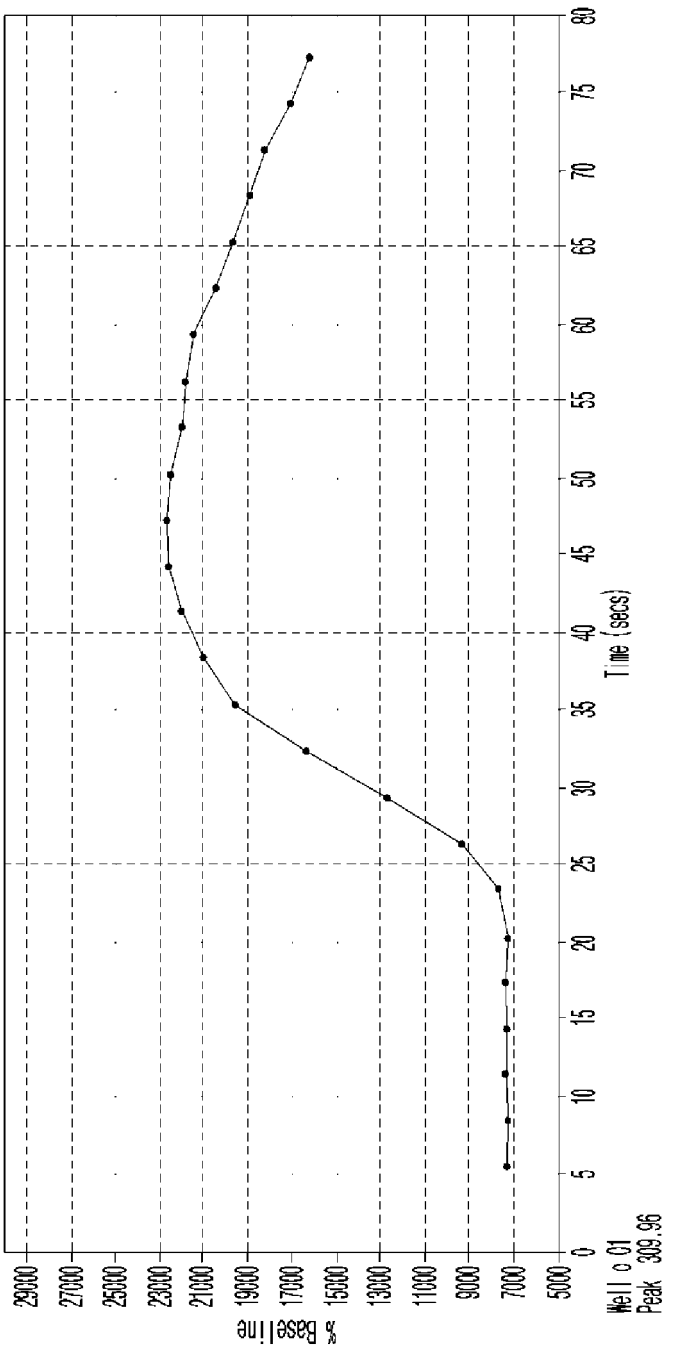
treating a pruritus-inducing substance to cells expressing MRGPR X1 (Mas-related G protein-coupled receptor), and culturing the cells (step 1); and
treating a candidate material to the pruritus-induced cells, and measuring the inhibition of MRGPR X1 activity (step 2).

10. The screening method of a compound for preventing or treating pruritus according to claim 9, wherein the pruritus-inducing substance of step 1 is chloroquine.

11. A screening method of an active material for preventing or treating pruritus, which comprises the following steps:

treating a pruritus-inducing substance to cells expressing hH1R (human Histamine 1 Receptor), and culturing the cells (step 1); and
treating a candidate material to the pruritus-induced cells, and measuring the inhibition of hH1R activity (step 2).

12. The screening method of a compound for preventing or treating pruritus according to claim 11, wherein the pruritus-inducing substance of step 1 is one or more substances selected from the group consisting of interleukin-1, cytokine, serotonin, acetylcholine, substance P, leukotrine and prostaglandin.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25000
23000
21000
19000
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15000
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11000
9000
7000
5000
0
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10
15
20
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55
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65
70
75
80
Time (secs)
Well c 01
Peak 309.96

Fig.1

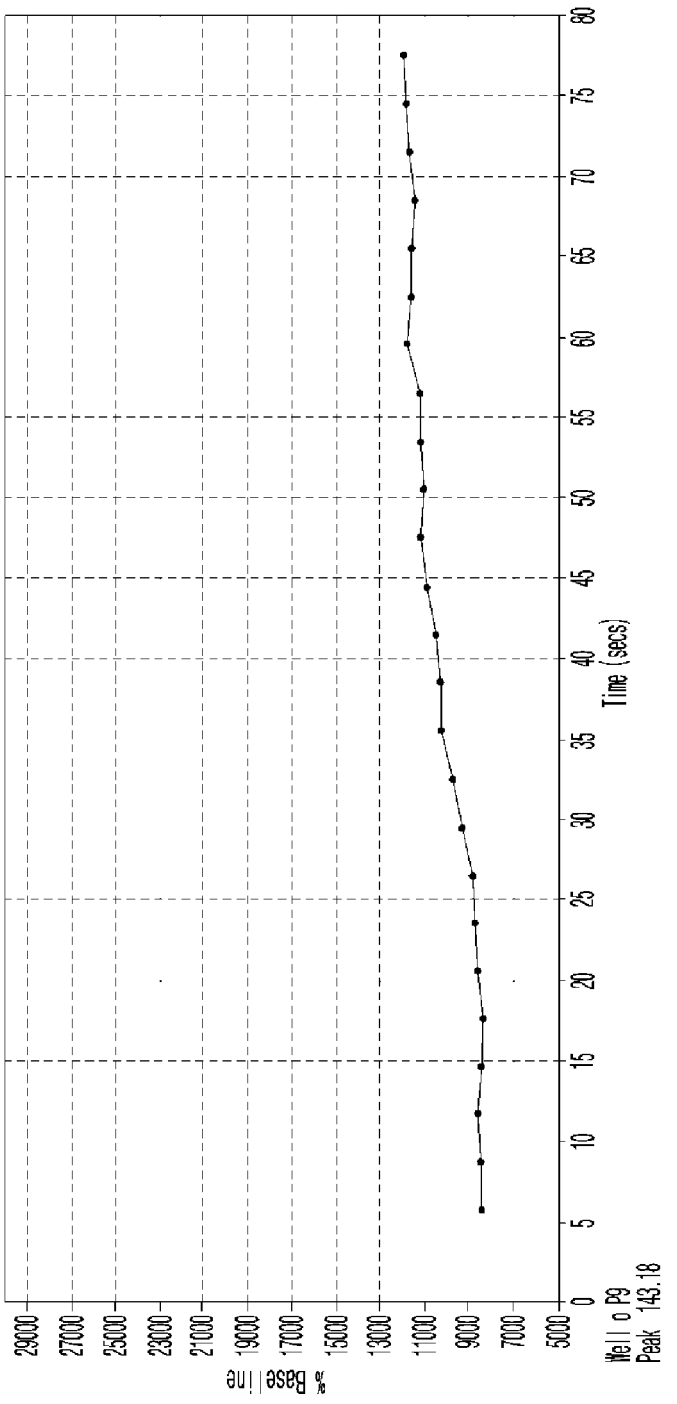
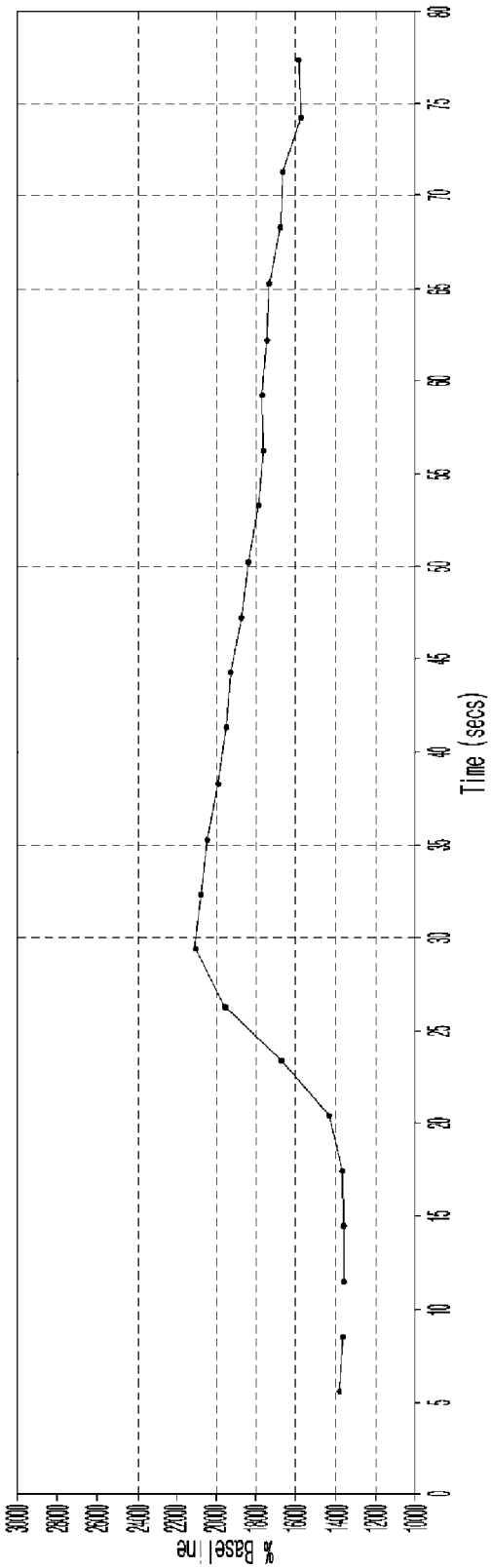


Fig.2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24000
22000
20000
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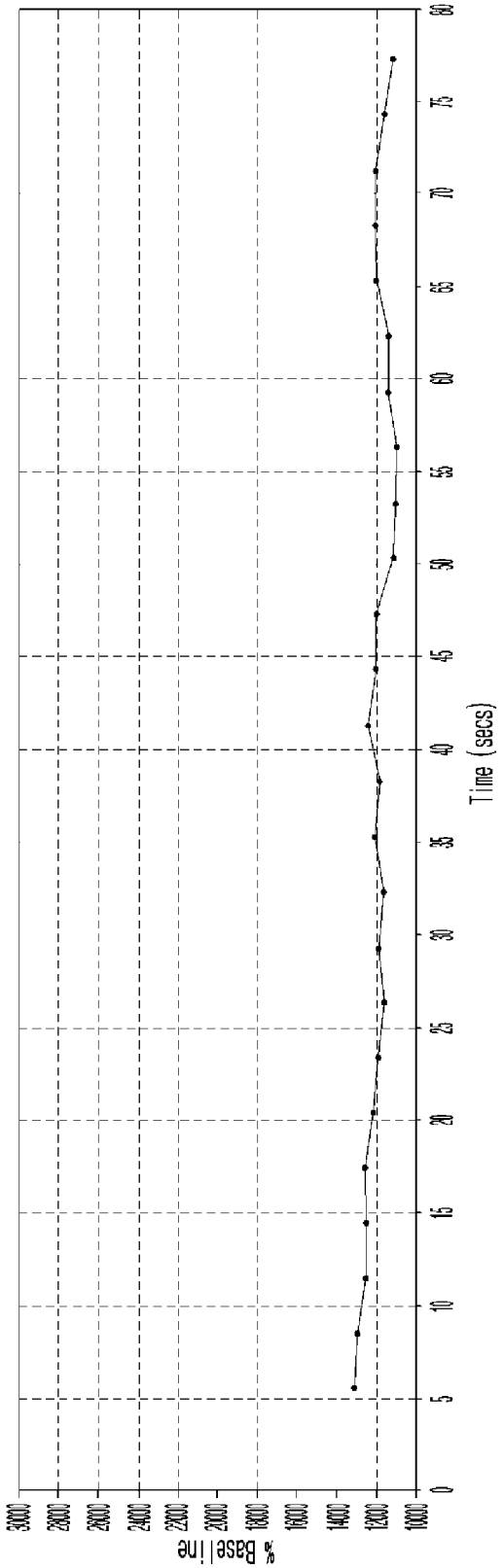
16000
14000
12000
10000
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
Time (secs)

Fig.3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14000
12000
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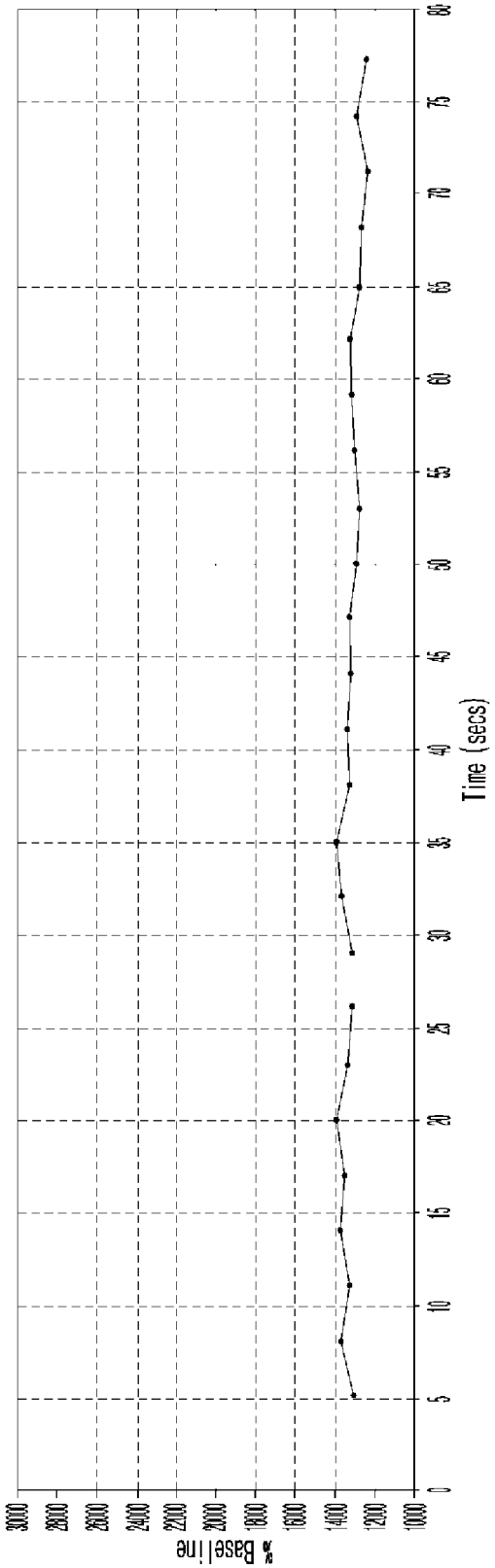
55
60
65
70
75
80
Time (secs)

Fig.4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26000
24000
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20000
18000
16000
14000
12000
10000
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
Time (secs)

Fig.5


200
150
100
50
0
# of scratch
Histamine
Pyrazole derivative + Histamine

Fig.6

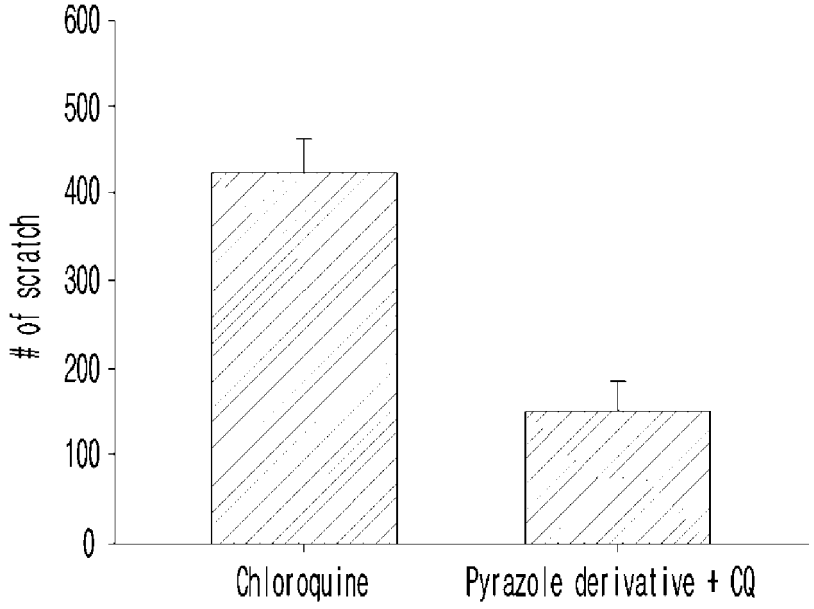
600
500
400
300
200
100
0
# of scratch
Chloroquine
Pyrazole derivative + CQ

Fig.7

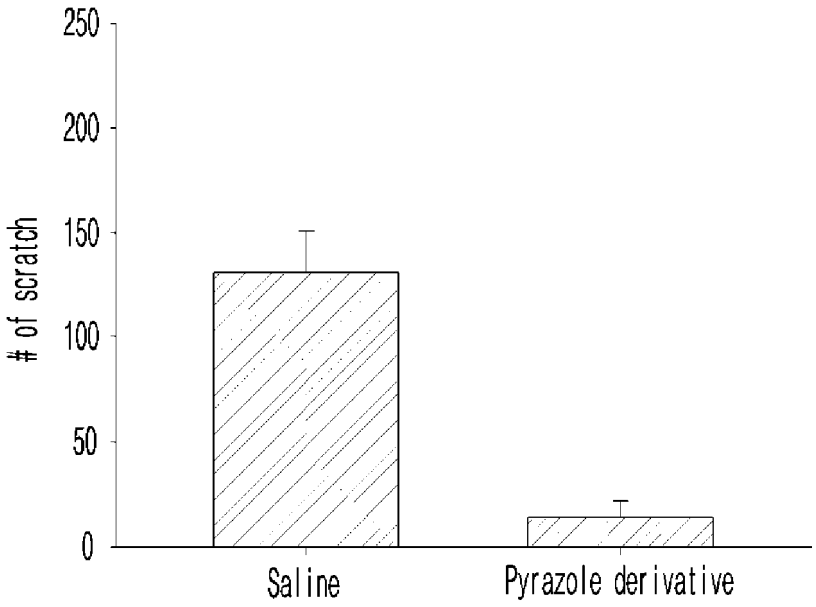

Fig.8

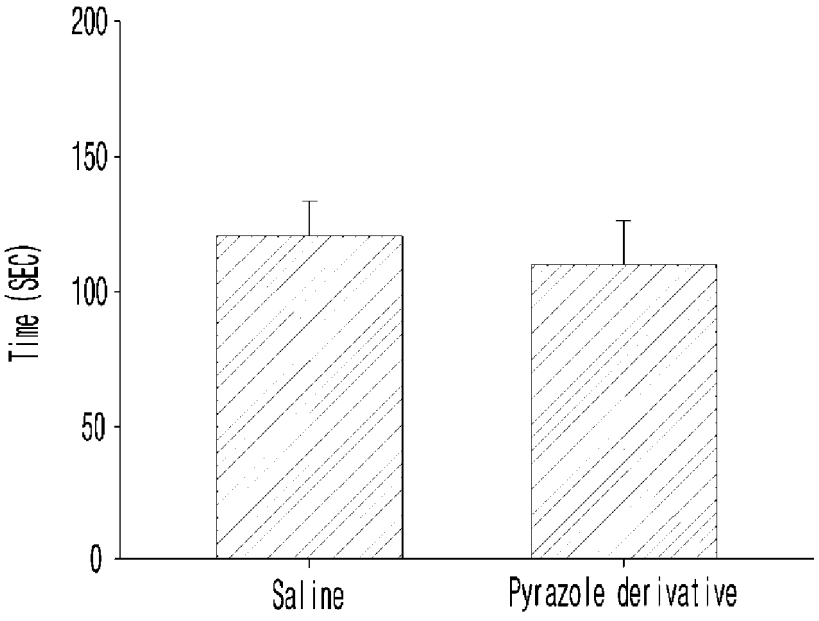

Fig.9

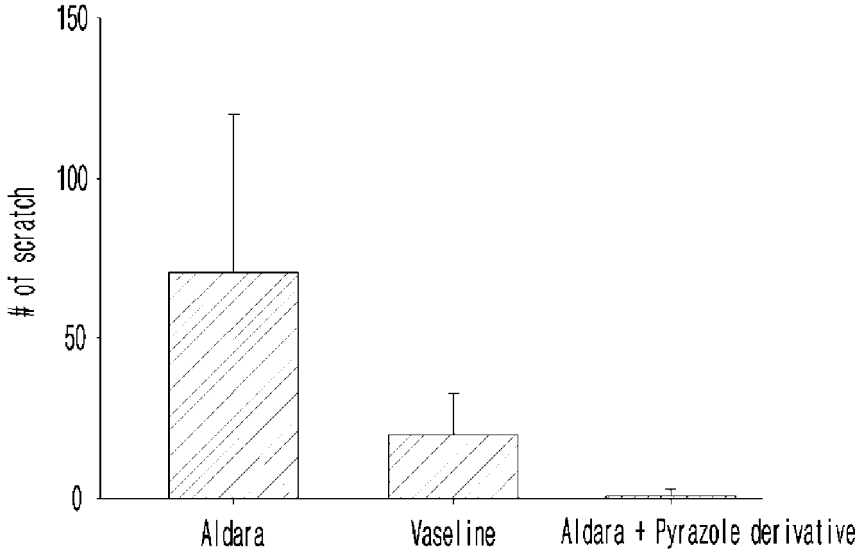
150
100
50
0
# of scratch
Aldara
Vaseline
Aldara + Pyrazole derivative

Fig.10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2018/002626

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/415(2006.01)i, A23L 33/10(2016.01)i, G01N 33/50(2006.01)i, G01N 33/68(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 31/415; C07D 231/12; C07D 403/02; C07D 453/02; A61K 31/439; A23L 33/10; G01N 33/50; G01N 33/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Utility models and applications for Utility models: IPC as above
Japanese Utility models and applications for Utility models: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS (KIPO internal), STN (Registry, Caplus), Google & Keywords: pruritus, pruritus, itch, pyrazole derivative, MRGPRX1 (mas-related G protein-coupled receptor X1), chloroquine, hH1R(human histamine 1 receptor), cytokine

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014-069554 A1 (TORAY INDUSTRIES, INC.) 08 May 2014 See abstract; claims 1, 5; and paragraph [0150].	1-8
X	BANDELL, M. et al., "Itching for Insight", Cell, 24 December 2009, vol. 139, no. 7, pages 1224-1226 See abstract; and figure 1.	9-10
X	OHSAWA, Y. et al., "The Role of Histamine H1 and H4 Receptors in Atopic Dermatitis: From Basic Research to Clinical Study", Allergology International, 2014, vol. 63, pages 533-542 See abstract; page 534, right column, lines 19-20; and table 2.	11-12
A	WO 98-41519 A1 (SMITHKLINE BEECHAM CORPORATION) 24 September 1998 See abstract; and claims 1, 7, 10.	1-12
A	JP 06-073014 A (ELF SANOFI) 15 March 1994 See claim 2; and paragraph [0008].	1-12

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 JUNE 2018 (15.06.2018)	**15 JUNE 2018 (15.06.2018)**
Name and mailing address of the ISA/KR Korean Intellectual Property Office Government Complex-Daejeon, 189 Seonsa-ro, Daejeon 302-701, Republic of Korea Facsimile No. +82-42-481-8578	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2018/002626

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See extra sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (January 2015)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR2018/002626

Patent document cited in search report	Publication date	Patent family member	Publication date
WO 2014-069554 A1	08/05/2014	NONE	
WO 98-41519 A1	24/09/1998	CA 2283797 A1	24/09/1998
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Form PCT/ISA/210 (patent family annex) (January 2015)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR2018/002626

Patent document cited in search report	Publication date	Patent family member	Publication date
		GR 3024470 T3	28/11/1997
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		HU 222577 B1	28/08/2003
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		JP 07-309841 A	28/11/1995
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Form PCT/ISA/210 (patent family annex) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2018/002626

The invention of group 1: claims 1 to 8 pertain to a pharmaceutical composition for preventing or treating chronic itch and a health functional food composition, both compositions containing a compound represented by chemical formula 1 as an active ingredient.

The invention of group 2: claims 9 and 10 pertain to a compound screening method for preventing or treating chronic itch by measuring activity inhibition of the Mas-related G protein-coupled receptor X1 (MRGPRX1).

The invention of group 3: claims 11 and 12 pertain to a compound screening method for alleviating or treating chronic itch by measuring activity inhibition of the human histamine 1 receptor (hH1R).

The technical feature shared by all of these claims is the compound for preventing or treating chronic itch. However, the compound corresponds to a technology disclosed in the international search report (WO 2014-069554 A1).

Therefore, since these claims have no common special technical feature which makes a contribution over the prior art under PCT Rule 13.2, there is no unity of invention.

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Non-patent literature cited in the description

- 2009 Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. *Cell,* 2009, vol. 139, 1353-1365 **[0005]**
- *Jpn J Pharmacol.,* March 2002, vol. 88 (3), 285-92 **[0093]**
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